

WHAT IS CLAIMED IS:

- 1. A method of establishing a clonal embryonic stem cell line capable of sustaining a phenotype of normal embryonic stem cells following at least eight months of *in vitro* culturing, the method comprising culturing an individual embryonic stem cell for at least eight months in a scrum-free medium, thereby establishing the clonal embryonic stem cell line capable of sustaining said phenotype of normal embryonic stem cells following at least eight months of *in vitro* culture.
- 2. The method of claim 1, wherein said individual embryonic stem cell is a human embryonic stem cell.
- 3. The method of claim 1, wherein the phenotype of normal embryonic stem cells is characterized by a normal karyotype, a non-increasing population doubling time selected from a range of 28 to 42 hours, a non-decreasing telomere length, non-decreasing telomerase activity and pluripotentiality.
- 4. The method of claim 3, wherein said non-increasing population doubling time is selected from a range of 33 to 37 hours.

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- 5. The method of claim 3, wherein said non-decreasing telomere length is selected from a range of 4 to 16 kb.
- 6. The method of claim 3, wherein said non-decreasing telomere length is selected from a range of 8 to 12 kb.
- 7. The method of claim 3, wherein said pluripotentiality is characterized by the capacity to differentiate into endodermal, mesodermal and ectodermal cells.
- 8. The method of claim 1, further comprising the step of obtaining said individual embryonic stem cell from a source selected from the group consisting of an embryonic stem cell culture, a blastocyst inner cell mass, a blastocyst, embryonic germ cells, an embryonic germ cell culture, an embryo and a fetus prior to said step of culturing.
- 9. The method of claim 8, wherein said step of obtaining said individual embryonic stem cell from said blastocyte inner mass is effected by:
 - (a) isolating a blastocyst;
 - (b) isolating cells from the inner cell mass of said blastocyst;
 - culturing said cells from the inner cell mass on mouse embryonic feeder fibroblasts, thereby generating an inner cell mass-derived cell mass;



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- (d) dissociating said inner cell mass-derived cell mass into dissociated cells;
- (e) culturing said dissociated cells on mouse embryonic feeder fibroblasts, thereby generating dissociated cell-derived colonies;
- (f) selectively harvesting from among said dissociated cell-derived colonies a colony with morphologically compact cells, cells with high nucleus-to-cytoplasm ratio and/or cells with prominent nucleoli; and
- (g) dissociating said colony with morphologically compact cells, cells with high nucleus-to-cytoplasm ratio and/or cells with prominent nucleoli into individual cells thereby obtaining said individual embryonic stem cell.
- 10. The method of claim 1, wherein said serum-free medium includes feeder fibroblasts.
- 11. The method of claim 10, wherein said feeder fibroblasts are murine.
- 12. The method of claim 10, wherein said feeder fibroblasts are embryonic.

- 13. The method of claim 1, wherein said serum-free medium includes 0.4 to 40 ng/ml bFGF.
- 14. The method of claim 1, wherein said serum-free medium includes 1 to 16 ng/ml bFGF.
- 15. The method of claim 1, wherein said serum-free medium includes 2 to 8 ng/ml bFGF.
- 16. The method of claim 1, wherein said serum-free medium includes 4 ng/ml bFGF.
- 17. A clonal human embryonic stem cell line being capable of sustaining a normal embryonic stem cell phenotype following at least eight months of *in vitro* culturing.
- 18. The clonal human embyonic stem cell line of claim 17, wherein said *in vitro* culturing is effected on mouse embryonic feeder fibroblasts in serum-free medium supplemented with basic fibroblast growth factor.
- 19. The clonal human embyonic stem cell line of claim 17, wherein the phenotype of normal embryonic stem cells is characterized by a normal karyotype, a non-increasing population doubling time selected from a range of





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28 to 42 hours, a non-decreasing telomere length, non-decreasing telomerase activity and pluripotentiality.

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- The clonal human embyonic stem cell line of claim 17, wherein 20. said non-increasing population doubling time is selected from a range of 33 to 37 hours.
- The clonal human embyonic stem cell line of claim 17, wherein 21. said non-decreasing telomere length is selected from a range of 4 to 16 kb.
- The clonal human embyonic stem cell line of claim 17, wherein 22. said non-decreasing telomere length is selected from a range of 8 to 12 kb.
- The clonal human embyonic stem cell line of claim 17, wherein 23. said pluripotentiality is characterized by the capacity to differentiate into endodermal, mesodermal and ectodermal cells.
- A clonal human embryonic stem cell line being capable of 24. sustaining a normal embryonic stem cell phenotype following at least twelve months of in vitro culturing.